Loss of Imprinting of the Insulin-like Growth Factor II Gene in Renal Cell Carcinoma

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Abstract

Loss of imprinting (LOI) has been implicated in the pathogenesis of embryonal malignancies as well as adult cancers. Insulin-like growth factor II (IGF2) gene is an imprinted gene, normally transcribed only from the paternal allele. We investigated allele-specific expression of the IGF2 gene in 22 cases of renal cell carcinoma (RCC), a common adult-onset renal tumor. Sixteen cases (72%) were informative for IGF2 gene expression, and 9 (56%) of these cases showed biallelic expression of the IGF2 gene. Additionally, in four cases with biallelic expression from which uninvolved kidney tissue was available, LOI of the IGF2 gene was also demonstrated in the normal tissue. All cases with LOI of IGF2 were low-stage tumors. LOI of the IGF2 gene in RCC was not associated with overexpression of IGF2 mRNA, whereas IGF2 overexpression was frequently observed in high-stage tumors. These results suggest that LOI of IGF2 predisposes to low-stage tumors, whereas IGF2 overexpression may have a role in RCC tumor progression.

Introduction

Genomic imprinting refers to allele-specific gene expression, which is dependent on parental origin. Several imprinted genes have been identified, including IGF2 and H19 (1–3), both of which are located on chromosome 11p15.5. Recently, disruption or LOI of these genes has been reported in some types of tumors, such as Wilms' tumor (3), hepatoblastoma (4), lung cancer (5), glioma (6), and esophageal cancer (7).

IGF2 is a 67-amino acid mitogenic peptide that plays an important role in fetal growth. IGF2 mRNA is also highly expressed in several types of tumors (8–10) and may act as an autocrine or paracrine growth factor. The regulation of the IGF2 gene is extremely complex, with four promoters reported to be under differential, developmental, and regional controls (11–13). Moreover, expression of IGF2 is normally imprinted, with only the paternal allele being expressed.

RCC is a common adult renal tumor in which loss of heterozygosity of the chromosome 11p15.5 region has been reported (14). Therefore, we investigated the allele-specific expression pattern of the IGF2 gene in RCC.

Materials and Methods

Patients and Tissue Samples. Renal tumor samples, together with uninvolved portions of kidney tissue if available, were obtained from 22 patients with RCC undergoing surgery at Osaka University Hospital. The grade and staging system of RCC were based on the criteria described by the American Joint Committee on Cancer (15). The samples were immediately frozen in liquid nitrogen and stored at −70°C until analysis.

DNA and RNA Preparation. Genomic DNA and total RNA were extracted from tumor tissues or normal kidney tissues of patients with RCC. One μg of total RNA was treated with 1 unit of DNase I (Life Technologies, Inc., Gaithersburg, MD) for 15 min at room temperature to eliminate DNA contamination, followed by heating for 10 min at 65°C to inactivate the DNase I.

RT-PCR and RFLP Analysis of IGF2. To identify informative cases that were heterozygous for a polymorphic site in the IGF2 gene, genomic DNA was amplified by PCR and digested with Apal, as described previously (16). The sequence of the primers used to amplify the IGF2 gene were as follows: IGF2-1 (sense primer), 5'CTTGAGCTTTGAGTCAAATTGG-3'; and IGF2-2 (antisense primer), 5'-CTCTGTGTGTTACTGGG-3'. The PCR was performed in a 50-μl volume with the PCR primers at a final concentration of 12.5 μM. After 5 min of initial denaturation at 94°C, amplification was performed for 40 cycles (94°C for 1 min, 65°C for 3 min, and 72°C for 3 min) using a Perkin-Elmer Thermocycler 480. The PCR products were digested with Apal (yielding either 236-bp fragment or 173- and 63-bp fragments), electrophoresed on a 3% agarose gel, and visualized with ethidium bromide.

If informative for the RFLP, cDNA was reverse-transcribed from total RNA (100 ng) treated with DNase I. The cDNA was amplified by PCR in the same manner as genomic PCR, and amplified cDNA was also digested with Apal. Because the amplified area of the IGF2 gene does not include an intron, RT-PCR was performed both with and without RT. As expected, no PCR products were observed when RT was not added, indicating that genomic DNA contaminants had been eliminated (data not shown).

Northern Blot Analysis of IGF2. Total RNA (20 μg) from RCC informative for IGF2 allelic expression was electrophoresed on a 1% agarose-formaldehyde gel and transferred to a nylon membrane (Hybond-N; Amersham Japan, Tokyo, Japan) in 20× SSC. Total RNA from placenta was used as a positive control. Filters were hybridized with a 32P-labeled IGF2 cDNA probe (a generous gift from Dr. Kazumasa Hashimoto, Osaka University Medical School), which contains exon 9 of the IGF2 coding sequence. Hybridization with a β-actin cDNA probe (CLONTECH, Palo Alto, CA) was performed as a control. IGF2 mRNA expression was not examined in cases 8, 9, and 10 due to insufficient material.

Results

Allele-specific Expression of IGF2. We investigated allele-specific expression of the IGF2 gene in 22 cases of RCC. Sixteen of 22 cases (cases 2, 3, 4, 6–13, 15, 16, 20, 21, and 22; 72%) had RFLPs that made them informative for IGF2 gene expression. Nine of these cases (cases 3, 4, 9–13, 15, and 20; 56%) demonstrated biallelic expression of the IGF2 gene (Fig. 1a). We then examined allele-specific expression of the IGF2 gene in noncancerous kidney tissues of the resected specimens in the four cases where it was available (cases 3, 12, 13, and 20), and all of the noncancerous kidney tissues also revealed LOI of the IGF2 gene (Fig. 1b; Table 1). We could obtain peripheral blood from three of these four cases (cases 12, 13, and 20; case 3 refused blood sampling). In these three cases examined
Alternative IGF2 imprinting in kidney cancer

Table 1: Clinicopathologic findings and IGF2 allelic expression in 22 cases with RCC

<table>
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<th>Sex</th>
<th>Age (yr)</th>
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<th>Grade</th>
<th>Cell type</th>
<th>Apal polymorphism</th>
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</table>

*TNM, tumor-node-metastasis; M, male; F, female; A, allele without Apal restriction site; B, allele with Apal restriction site; AB, biallelic expression; —, uninformative or not done.

Discussion

In this study, we demonstrate that LOI of IGF2 occurred in 9 (56%) of 16 informative RCC cases, a common adult renal tumor. In contrast, the H19 gene, located in the same region of chromosome 11 as IGF2, was imprinted in all cases with RCC (data not shown). This implies that allelic expression of IGF2 and H19 are independent events. Thus, the regulation of these genes in adult renal tumors may be different from that observed in Wilms' tumors, in which reciprocal expression of IGF2 and H19 genes has been observed (3).

Although LOI (biallelic expression) of the IGF2 gene theoretically suggests that there will be a 2-fold increase in IGF2 expression, our
data indicate that LOI does not lead to IGF2 overexpression. However, high levels of IGF2 mRNA were detected in some cases (cases 2, 3, 16, and 22) in whom IGF2 expression was imprinted. These results may be due to the complexity of IGF2 expression, which is governed in a tissue- and stage-specific way by four promoters (P1–P4), leading to the synthesis of a family of transcripts that all encode a pre-pro-IGF2 (8). Moreover, P2, P3, and P4 promoters have been found to display monoallelic paternal activity, whereas only IGF2 transcripts from P1 have been demonstrated to be induced from both parental alleles. Because we have not examined the difference in mRNA expression between tumor and normal tissue (due to insufficient samples), we cannot definitively exclude the possibility that LOI of the IGF2 gene is due to a switch in promoter usage.

Interestingly, neighboring normal kidney tissue also showed LOI of the IGF2 gene in the four cases evaluated in the present study. Fortunately, we could obtain peripheral blood from three of these four cases (cases 12, 13, and 20; case 4 refused blood sampling). Contrary to the situation in normal kidney tissues, imprinting of IGF2 gene was maintained in peripheral blood lymphocytes, in whom LOI of the IGF2 gene was found to be a tumor-specific event (16). Although the number of cases were small, our findings suggest that, at least in RCC, LOI is a genetic alteration that is organ-specific rather than tumor-specific.

IGF2 overexpression is a common feature in some tumors and is hypothesized to function as a growth factor or mitogenic factor (8–10). In RCC, no definitive growth-promoting factor has thus far been identified, but our demonstration that IGF2 overexpression was observed in high-stage cases (cases 2, 16, and 22) suggests that IGF2 may act as a growth factor in some RCCs.

Although the number of cases in this study is small, LOI of the IGF2 gene was more frequently observed in low-grade and low-stage cases and neighboring normal renal tissue than in high-stage tumors. Moreover, LOI of the IGF2 gene did not lead to overexpression, whereas IGF2 mRNA was highly expressed in high stage tumors in which IGF2 imprinting was maintained. These results suggest that LOI of the IGF2 gene is a genetic abnormality that may predispose to low-grade tumors, whereas IGF2 overexpression may have a role in tumor progression.

Acknowledgments

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References

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